

THE GOVERNMENT OF THE PHILIPPINE ISLANDS
DEPARTMENT OF PUBLIC INSTRUCTION
BUREAU OF AGRICULTURE
FREDERIC W. TAYLOR, DIRECTOR OF AGRICULTURE

BULLETIN NO. 28

**THE
MECHANICAL TRANSMISSION OF
SURRA BY TABANUS STRIATUS**

BY

**M. BRUIN MITZMAIN, M. S.
VETERINARY ENTOMOLOGIST**

UNDER THE DIRECTION OF

**ARCHIBALD R. WARD, B. S. A., D. V. M.
CHIEF VETERINARIAN**

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THE GOVERNMENT OF THE PHILIPPINE ISLANDS,
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LETTER OF TRANSMITTAL.

MANILA, *September 15, 1913.*

SIR: I have the honor to transmit herewith, and to recommend for publication as a bulletin of this Bureau, manuscript by M. Bruin Mitzmain, veterinary entomologist, entitled "Mechanical Transmission of Surra by *Tabanus striatus*." This paper appears also in the Philippine Journal of Science, Section B (1913), Volume VIII, page 223.

Very respectfully,

A. R. WARD,
Chief Veterinarian.

H. T. EDWARDS,
Acting Director of Agriculture, Manila, P. I.

Publication recommended.

H. T. EDWARDS,
Acting Director.

Publication authorized.

NEWTON W. GILBERT,
Secretary of Public Instruction.

THE MECHANICAL TRANSMISSION OF SURRA BY *TABANUS STRIATUS*

By M. BRUIN MITZMAIN

(From the Veterinary Division,¹ Bureau of Agriculture, Manila, P. I.)

The Tabanidæ have hitherto not been investigated in connection with the transmission of surra in the Philippine Islands, as *Stomoxys calcitrans* has been the only carrier generally suspected. Recent work² has, however, practically eliminated the latter fly from further consideration as an important factor in surra dissemination. The investigation of which this is a preliminary note has been conducted during the past year in the veterinary research laboratory at Alabang, Rizal Province, Luzon, with tabanid flies, which were for the most part bred from the egg; in some instances the flies were obtained from larvæ taken from their aquatic habitat; and in a few instances captured adult flies were employed. In no place has the transmission of any trypanosome infections through the bite of tabanid flies been proved with flies bred in the laboratory.

The geographical and seasonal distribution of *Tabanus striatus* have been recorded in a recent article, and the status thereof marks this species as preëminently the most formidable blood-sucking fly of the Philippines.

In the present series *Tabanus striatus* was used in experiments on the direct transmission of surra. The flies were first allowed to bite an infected guinea pig or horse for not more than one minute, usually forty-five seconds; they were then transferred to a healthy animal and allowed to complete the meal without interruption. An interval of from five seconds to three minutes intervened during the transfer from the infected to the healthy animal.

In every instance the healthy animals used in the experiments were proved to be free from trypanosomes by rigorous quaran-

¹ Archibald R. Ward, chief.

² *Philippine Journal of Science*, Sec. B (1913), 7, 475.

tine and frequent microscopic examination of their blood. The mule and the horses employed in these experiments were kept for from six to eight months in the screened stable prior to their use for exposure to fly bites or blood inoculation to test the pathogenic nature of the trypanosome involved. The temperatures of these animals were registered morning and evening during the period of quarantine, preceding and following each experiment.

Monkeys, guinea pigs, and rabbits utilized in the experiments were quartered previously in fly-screened cages and declared surra free after ninety days, during which time blood examinations were made regularly once or twice weekly prior to the experiments.

Table I gives the data of these experiments.

TABLE I.—*Experiments on the mechanical transmission of surra by Tabanus striatus.*

| Date of experiment. | Trypanosomes in the infected host. | Flies employed. | Healthy animal used. | Result of experiment. |
|---------------------|------------------------------------|-----------------|----------------------|--|
| 1912 | | | | |
| Nov. 21 | Swarming | 1 | Guinea pig 87 | Negative. |
| 22 | Moderate | 1 | Guinea pig 92 | Negative. Subsequently reacted to surra by blood inoculation. |
| Dec. 12 | do | 1 | Guinea pig 111 | Negative. |
| 26 | do | 2 | Guinea pig 93 | Do. |
| Nov. 21 | Numerous | 1 | Monkey 5 | Do. |
| 22 | Swarming | 1 | Monkey P | Do. |
| 30 | Numerous | 1 | Monkey D | Do. |
| Dec. 1 | do | 1 | Monkey S | Do. |
| 28 | do. ^a | 3 | Monkey L | Positive on the eighth day. Dead Jan. 22. Blood produced surra in 2 guinea pigs and 1 horse. The latter died Mar. 1, 1913. |
| 1913 | | | | |
| Jan. 1 | Scanty | 2 | Monkey D | Negative. |
| 26 | Numerous | 2 | Horse 66 | Do. |
| 27 | do | 2 | Horse 69 | Do. ^c |
| 28 | Swarming | 1 | Horse 277 | Do. ^c |
| 30 | Numerous | 1 | Horse B-120 | Do. |
| Feb. 2 | Swarming ^b | 2 | Horse 50 | Positive on eighth day. One mule, 2 monkeys, and 2 guinea pigs reacted to inoculation of blood of horse 50. |
| 6 to 10. | Scanty to moderate. ^b | 6 | Horse 342 | Positive on ninth day. Disease reproduced by blood inoculation into 2 monkeys and 2 guinea pigs. |

^a Infected guinea pig used.

^b Infected horse used.

^c Reacted subsequently to inoculation of blood of infected horse.

The three positive experiments are described in detail as follows:

The experiment in which monkey L became infected was conducted with flies bred from eggs. The source of this strain of surra was carabao 3182 which had been infected with surra for nearly one year previous to the experiment. Blood of this animal was inoculated into guinea pig 119 which proved infectious in eight days, and was used for the present experiment, December 28, when its blood showed numerous trypanosomes. Three flies were applied individually from tubes to guinea pig 119 and allowed to feed from forty-five seconds to one minute and thirty seconds. They were then transferred to monkey L, after intervals of twenty seconds to three minutes, and allowed to feed until satisfied. The flies fed on the latter animal five, sixteen, and twenty-one minutes, respectively.

From December 28 to January 8 no reaction was noted. The first high temperature, $40^{\circ}.1$ C., occurred on the evening of January 8, accompanied by a few trypanosomes in the peripheral circulation. The presence of trypanosomes continued daily, moderate to swarming in numbers, with several febrile periods until the animal's death on January 22, 1913.

Blood from the heart of monkey L was inoculated into horse 343 and guinea pigs 101 and 102. The latter showed infection upon the eighth and ninth days, respectively. Horse 343 had an abnormal temperature and a moderate number of trypanosomes in its blood upon the seventh day. The animal died March 1 with surra. At autopsy there was observed a general emaciated condition and enlargement of the spleen. The splenic pulp contained enormous quantities of trypanosomes as did the heart's blood.

Horse 343 was also used as the blood donor in transmission experiments in which tabanids infected two other horses, namely, 50 and 342. The latter experiments were made in order to verify the previous one, in which a monkey contracted the disease. In only the first of the latter experiments were bred flies used. Two flies were permitted to bite horse 343 at a time when its blood was swarming with trypanosomes. The flies were interrupted in their biting in from forty to forty-five seconds and transferred to healthy horse 50. The infected flies bit after intervals of five to fifteen seconds and were permitted to complete the feeding on horse 50. The latter was replaced in the fly-screened stable and examined daily. The initial rise of temperature was noted upon the eighth day, February 10, when a few surra organisms were found in the animal's blood. On

the day following, horse 50 showed numerous trypanosomes in its blood and high temperature. Blood from this horse was inoculated into a mule, 2 monkeys, and 2 guinea pigs. The mule reacted with the usual symptoms after an incubation period of six days. Both monkeys had an incubation period of five days, and died of surra on the fourteenth and the fifteenth day, respectively. The 2 guinea pigs likewise became infected.

The second experiment was conducted with captured flies, infected horse 343 being used in this experiment. From February 6 to February 10, six flies in all were allowed to bite healthy horse 342 after they contaminated their proboscides with the blood of horse 343. The blood of the latter contained numerous trypanosomes upon only one occasion during the experiment; at other times the trypanosomes were scanty or moderate in numbers. The interval between the biting of the infected and the healthy horse was never more than twenty seconds, and the meal was completed in four to eleven minutes on horse 342. An incubation period of nine days following the last bite elapsed before the healthy horse showed evidence of infection from the bites of the contaminated flies. On the evening of February 19 the temperature of horse 342 rose to 41°.1 C., and the blood showed a moderate number of trypanosomes. Upon the following day, when the trypanosomes were more numerous, blood from this animal was inoculated into 2 monkeys and 2 guinea pigs. The monkeys showed the first signs of infection on the seventh day and the guinea pigs on the eighth and ninth days. The 2 monkeys and the 2 guinea pigs were alive, but still infected on March 10, 1913.

BITING EXPERIMENTS WITH ANIMALS IN A LARGE CAGE

An effort was made to induce flies to feed on healthy and infected animals kept together in a large screened cage. The results were negative, the flies dying in a few days when kept within the inclosure. The animals used were 2 surra-infected and 1 healthy carabao. The latter was separated from the others by a coarse-meshed wire partition. The flies were introduced daily into the common inclosure and were given ample opportunity to bite the animals exposed. From November 9 to December 22, 1911, 2,087 female tabanids were liberated in the cage. The animals were examined daily, and after the experiment the healthy animal was removed and observed. Fourteen months have elapsed and the exposed carabao remains normal. Two guinea pigs inoculated with its blood were alive and negative on April 12, 1913.

HEREDITARY TRANSMISSION

As a precautionary measure it was thought advisable to eliminate the possibility, however remote, of the existence of hereditary transmission of trypanosomes in these flies.

In one experiment of this nature 74 flies were tested during two weeks after the emergence of the lot by allowing them to bite a healthy monkey. The eggs from which these flies developed had been laid August 14 by a fly which had fed twice on a monkey infected with surra and whose blood was swarming with trypanosomes. The following table contains the data resulting from allowing flies of this lot to feed on a healthy monkey. Monkey 5, which was examined daily during the experiment, showed no signs of infection and remained healthy until April 12, 1913.

TABLE II.—*Experiments to test the hereditary transmission of surra infection in Tabanus on monkey 5; results negative.*

| Date. | Flies tested. | Average time of feeding. |
|-------------|---------------|--------------------------|
| | | Min. |
| Nov. 5..... | 1 | 4 |
| 7..... | 3 | 7 |
| 8..... | 3 | 7 |
| 9..... | 4 | 9 |
| 10..... | 3 | 6 |
| 12..... | 5 | 6 |
| 13..... | 10 | 9 |
| 14..... | 9 | 10 |
| 15..... | 6 | 8 |
| 16..... | 8 | 6 |
| 17..... | 4 | 7 |
| 18..... | 7 | 6 |
| 19..... | 6 | 4 |
| 20..... | 5 | 4 |

AN ATTEMPT TO TRANSMIT SURRA BY MEANS OTHER THAN BITING

It was observed that many flies of both sexes in feeding supplemented the sucking of the labium with the lapping of the spongy labellum. Usually these processes are independent, but not infrequently the female in attempting to bite the host will lap up any moisture present preparatory to inserting its proboscis. When an abraded surface is presented the majority of flies of this species are capable of nearly filling the stomach with blood without the aid of the proboscis. This has been observed in numerous instances in flies used in biting experi-

ments, but has never been seen in flies attacking animals in the natural state.

Considering that there might be a remote possibility of conveyance of infection through this peculiarity in feeding habits, five experiments, based on this hypothesis, were attempted. The technique was the same in all of the 5 guinea pigs used. A highly infected guinea pig was used to contaminate the flies. A portion of the skin of infected and healthy animals was abraded with a razor and the flies applied individually from tubes. The fly was permitted to lap the blood from the abrasion on the infected animal for a minute or less and then transferred immediately to the healthy animal, where it was induced to apply its labellum for five to ten minutes.

TABLE III.—*Attempts to transfer infection by the fly's labellum. Results negative.**

| Date. | No. of guinea pig. | Flies applied. |
|---------------|--------------------|----------------|
| Nov. 21 | 85 | 3 |
| Nov. 22 | 91 | 2 |
| Do | 110 | 2 |
| Nov. 23 | 104 | 4 |
| Nov. 24 | 123 | 3 |

* The experiment with guinea pig 110 was checked by subsequent inoculation to which the animal became infected.

The results of all the trials were negative, although it was ascertained that typical trypanosomes were present upon the labellum of one of the flies and in the stomach of another fly immediately after the experiment.

These experiments were supplemented by an experiment attempting to transfer infection to abrasions by the pulvilli of the contaminated fly. This also resulted negatively.

LENGTH OF TIME TABANUS STRIATUS HARBORS TRYPANOSOMES

An effort was made in this series of experiments to determine the maximum length of time surra organisms remain alive in the gut of the fly. The fly was fed in each instance on a guinea pig showing numerous trypanosomes in its blood. As noted in Table IV trypanosomes indistinguishable from surra organisms were found in suspensions from flies up to thirty hours after biting a sick animal. Beyond ten hours, inoculations of infected flies into susceptible animals proved negative.

TABLE IV.—*Inoculations with suspensions of flies fed on infected animals.*

| Ex- peri- ment No. | Animal inoculated. | Flies em- ployed. | Inter- val after feeding of fly on in- fected animal. | Presence of trypanosomes in the fly. | Result. |
|-----------------------------|----------------------|-------------------------|--|--|-----------|
| | | | <i>Hours.</i> | | |
| 1 | Guinea pig 86 | 1 | 6 | Positive | Positive. |
| 2 | Guinea pig 89 | 1 | 10 | do | Do. |
| 3 | Guinea pig P | 1 | 24 | do | Negative. |
| 4 | Guinea pig 131 | 1 | 26 | do | Do. |
| 5 | Guinea pig 130 | 1 | 30 | do | Do. |
| 6 | Rabbit | 1 | 30 | do | Do. |
| 7 | Guinea pig 97 | 1 | 48 | Negative | Do. |
| 8 | Guinea pig 98 | 1 | 48 | do | Do. |
| 9 | Guinea pig R | 1 | 96 | do | Do. |

* The dejecta from this fly were found swarming with trypanosomes two and one-half hours after the fly bit the sick animal.

SUMMARY.

1. *Tabanus striatus* Fabricus for the first time recorded has been found to play a rôle in the transmission of surra. Bred horseflies have been employed for the first time in such experiments. Errors resulting from the use of naturally infected wild flies have thus been eliminated.

2. Three experiments were successful in the direct or mechanical transmission by "interrupted" feeding when only a short interval was allowed between the bites on infected and healthy animals. In 16 experiments the minimum number of flies with which the infection could be transmitted was 2.

3. Trypanosomes of surra were not found to be transmitted hereditarily in *Tabanus striatus* Fabricus.

4. The contaminated labellum of the fly does not appear to be a factor in the conveyance of infection.

5. The maximum length of time that *Trypanosoma evansi* has been demonstrated microscopically in the gut of this species of fly after feeding on infected blood is thirty hours; the organisms were found in the fly's dejecta two and one-half hours after biting the infected animal; and suspensions of flies, when injected subcutaneously, were found infective for animals for a period of ten hours after the flies had fed on virulent blood.

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A limited number of the following-named bulletins and circulars are available for free distribution. Applicants for these should state the amount of land which they have under cultivation, kinds of crops planted, etc., together with such information as will enable the Bureau of Agriculture to make an intelligent distribution of these publications. All communications should be addressed to the Director of Agriculture, Manila, P. I.

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